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(71) Applicant: AGRI-TEK, INC. [US/US]; 6662 Morganton Road, Greenback, TN 37742 (US).

(72) Inventors: MILNE, Christopher, G.; 6662 Morganton Road, Greenback, TN 37742 (US). SHELBY, Paulus, P.; 3115 B Marion Drive, Knoxville, TN 37918 (US).

(74) Agent: MARKVA, Neil, F.; 8322-A Traford Lane, Springfield, VA 22152 (US).

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(57) Abstract

The present invention relates generally to the method for the production of liposomal microencapsulated products to be used for agricultural formulations. More specifically, a new method of production of liposomal microencapsulated is disclosed for active agents such as pesticides. A lecithin is mixed with an organic solvent in a certain proportion so as to provide solutions with varied levels of solubilized lecithin. The particular solvent being used will depend on the amount of active agent (AA) desired in the final solution. The formulation of the lecithin/organic solvent mixture is then allowed to settle. After settling, the top layer is separated and saved, while the bottom layer is discarded. An AA is then added to form a concentrate that is added to water for vesicle formation.

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METHOD OF PREPARING A PREDETERMINED ACTIVE AGENT STOCK SOLUTION FOR LIPOSOMAL MICROENCAPSULATION OF ACTIVE AGENTS FOR AGRICULTURAL USES

RELATED APPLICATIONS

This international application corresponds to copending U.S. Application Serial No. 08/248,480 filed May 24, 1994, which is a continuation-in-part of copending U.S. Application Serial No. 08/067,530 filed May 25, 1993, which is a continuation-in-part of U.S. Application Serial No. 07/737,202 filed July 29, 1991.

FIELD OF THE INVENTION

The present invention relates generally to the method for the production of liposomal microencapsulated products. More specifically the invention relates to a new method of producing liposomal microencapsulated agricultural active agents such as pesticides including herbicides, fungicides, insecticides, bactericides and other compounds.

BACKGROUND OF THE INVENTION

Pesticides such as herbicides, fungicides, insecticides, bactericides and other active agents and compounds are applied periodically in the home, agriculture, and other places and can be dangerous to humans. Farmers, however, still need to spray their crops and animals with these active agents and compounds. To this end, there have been several unsuccessful attempts to provide a method of delivering these compounds in a manner that is safe, effective, and economical, as well as environmentally acceptable.

Various encapsulating techniques have been tried with agricultural active agents with results considered insufficient to justify replacement of existing agricultural formulations. Nothing in the prior art either suggests, teaches, or discloses the use of liposomal microencapsulation techniques to active agents such as pesticides in agricultural formulations.

Liposomal microencapsulation is known in the pharmaceutical industry and has five steps. Ethanol (95%) is mixed in a particular proportion with high grade soybean lecithin containing 50% phosphatidylcholine (PC). The ethanol soybean lecithin mixture is agitated until the PC and other soluble portions of the lecithin have been dissolved into the ethanol. The mixture is then allowed to stand for a period of time, so that the insoluble portions can settle to the bottom of the container, and the top becomes a clear amber color. The top portion is then drawn off and saved. The bottom sludge is discarded. A certain amount of water is added to the mixture, followed by a predetermined amount of ethanol.

The steps followed up to this point result in a basic "stock" solution that is mixed with an active agent (AA) of choice in the pharmaceutical industry. The next step is the addition of a preselected AA to the stock solution. The final step is to then add the preselected AA solution to water, thereby effecting formation of the microcapsules or vesicles.

This known pharmaceutical method is limited to the use of 95% ethanol as a solvent and a high grade (50% PC) granular soybean lecithin as the only lipid source. This procedure produces a dilute solution because of the low amount of lecithin used and the addition of extra ethanol and water. This is acceptable in the medical field because dose rates are very low, thus requiring low loading potentials. This

known process is not acceptable in other fields, however, which require higher loading potentials such as in agricultural formulations used for pesticides.

Ethanol is the only solvent that is usable in the pharmaceutical industry and that is a problem in many agricultural applications, because not all agricultural compounds are soluble in ethanol. Furthermore, ethanol is a highly flammable solvent, and expensive EPA regulations on the proper packaging of flammable materials make ethanol impractical to use in most agricultural uses necessitating a less flammable solvent system. For these reasons, there is no suggestion in the pharmaceutical use of ethanol to use ethanol in agricultural formulations.

The mere extraction of lecithin from animal sources such as egg yolks does not relate to the agricultural industry. Japanese Patent No. C87-154187 discloses the extraction of lecithin from egg yolks. It states that the uses and advantages are for food, drugs and toiletries. Japanese Patent Nos. C88-116693 and C89-086119 disclose methods of further extraction and purification of phosphatidylcholine (PC) from egg lecithin. These patents disclose the use of egg lecithin as an emulsifier for food, drugs, and toiletries, but do not suggest making liposomes or liposomal carrier systems. These Japanese patent references specify a method of extraction and purification of PC from egg lecithin.

As determined in the pharmaceutical industry, animal or egg lecithin contains a higher percentage of saturated fatty acid side chains, which impart a more rigid gelatinous quality to resulting liposomes when used for liposomal encapsulation of drugs. In turn, there is a slower, more extended release rate of the entrapped drugs. This characteristic is advantageous for drug delivery systems but is not desirable in agricultural applications of pesticides.

where there may be a risk of causing chemical residue problems.

The average price for high purity (99%) animal PC at $75.00 per 100 milligrams is $340,194.00 per pound. The average price for high purity (99%) egg PC at $76.00 per 100 milligrams is $34,473.00 per pound. Low purity (60%) PC egg PC at $0.68 per 100 milligrams is $308.44 per pound. Soybean lecithin with PC content between twenty percent (20%) to forty percent (40%) can be purchased for under $10.00 per pound.

Canadian Patent No. 834,472 discloses the process of extracting PC from crude vegetable oils using monoglycerides to aid the process. This reference discloses varying the levels of the monoglycerides and different ways of using the monoglycerides in the process. The reference does not mention, suggest, teach, or disclose liposome formation or, more specifically, liposomal encapsulation of active agents for agricultural uses. Its use is strictly for food additives, bakery uses, cosmetics, and a one word mention of a medical use.

Active agents of particular interest in the agricultural industry are pesticides, which is a generic term for herbicides, fungicides, bactericides, and insecticides. Other agricultural active agents include dyes and stains. It has been discovered in this invention that the key to encapsulating such active agents for agricultural applications is the amphipathic material known as lecithin and, more specifically, plant lecithin.

In the American Oil Chemists' Society book entitled *Lecithins* and edited by Bernard F. Szuhaj and Gary R. List, at page 289, author Y. Pomeranz states that the "term 'lecithin' is the commercial or popular name for a naturally occurring mixture of similar compounds more accurately identified as phosphatides or phospholipids. The principal

components of the natural mixture are phosphatidylcholine, phosphatidylethanolamine, inositol phosphatides and related phosphorus-containing lipids."

At page 1 of the book, *Lecithins*, author C. R. Scholfield says that "[I]n modern usage, lecithin generally refers to a complex, naturally occurring mixture to phosphatides obtained by water-washing crude vegetable oil and separating and drying the hydrated gums. In addition to the phosphatides, such products contain triglycerides and other substances that are removed in an emulsion with gums. Soybean lecithin, the most common commercial product, has been reported to contain 25-35% triglycerides and smaller amounts of other nonphosphatide materials."

Commercially available plant lecithin is composed of the phospholipids called phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidic acid (PA), carotenoid, and, depending on the grade of the plant lecithin, varying levels of oils, triglycerides, fibrous materials and, in some cases, additives and surfactants. Commercial lecithins are available in dry granular, liquid, gel, paste, and powder forms.

The invention is limited to the use of plant lecithins as contrasted with animal lecithins that are obtained from animal sources such as egg yolks. As is well known, plant lecithins are found in soybean oil, cottonseed oil, canola oil, wheat oil, kelp, peanuts, and sunflower seeds.

At pages 185-188 and 195 of the book *Lecithins* published in 1985, authors J.C. Schmidt and F.T. Orthoefer discuss nonfood uses of lecithin. A known miscellaneous function of lecithin is as a liposomal encapsulating agent. Among nonfood applications, however, lecithin is used as a liposomal encapsulating agent only in the pharmaceutical industry. The authors discuss agricultural and agriproduct processing uses of lecithin with a particular small section

concerning pesticides.

Nothing in the book, *Lecithins*, teaches, discloses, or suggests the use of plant lecithin as the key to using liposomal encapsulating techniques for encapsulation of active agents such as pesticides for agricultural applications. Moreover, there is nothing in any prior art to suggest, teach, or disclose the liposomal encapsulation of active agents for agricultural applications.

It is known that pharmaceuticals and drugs are applied in low doses such as in milligrams and parts per million for human consumption. In comparison, pesticides are applied in known quantities measured in terms of pounds of active agent per acre of crop. All commercially available agricultural chemicals such as pesticides have known formulations for effecting their desired results. Some known pesticides contain as much a 48% and even up to 72% active agent in their known formulations.

The pharmaceutical application of lecithin, consequently, does not offer any help in using plant lecithin as a liposomal encapsulating agent in agricultural applications that require loading high concentrations of active agent into the initial stock solution composition of the invention. For example, the initial stock solution used in pharmaceutical applications uses a very low lipid content so that large amounts of active agent cannot be loaded into it.

It is known that PC is the material in plant lecithin that actually does the encapsulating in the liposomal microencapsulation process. The molecule of PC has a phosphate head with a choline moiety and some fatty acid chains that form a tail portion. The fatty acid chains are nonpolar and therefore repel water. The phosphate head of the PC molecule attracts water. When placed in water, the molecules coalesce so that the molecule tails are directed one way and the heads another to produce the vesicle formation of the

liposomal encapsulation technique.

PURPOSE OF THE INVENTION

The primary object of the invention is to provide a method of liposomal microencapsulation that will lead to EPA and FDA approval of encapsulated active agents such as pesticides, which are readily available at low cost.

Another object is to provide an excellent protective barrier for pesticides and other active agents from ultraviolet radiation, thereby enhancing the active life of ultraviolet-degradable active agents in agricultural uses.

A further object is to provide a method that will encapsulate more active ingredient per unit of volume than is available in any liposomal encapsulation of the prior art.

Another object of the invention is to provide a safe delivery system of pesticides for agricultural uses that does not require specialized handling and storage facilities.

A still further object of the invention is to provide an encapsulation method that will produce an encapsulated product having a slow release of the encapsulated compound.

Another object of is to produce an encapsulated active agent that gives protection against microbial breakdown if the active agent is applied to the soil and that allows for the delivery of the active agent, which will not be removed by rain or irrigation.

Another object of the present invention is to provide a process of liposomal microencapsulation that requires fewer and more cost effective ingredients and is, therefore, less expensive and time consuming to produce.

A further object of the present invention is to provide a method of encapsulation that produces an encapsulated active agent, which binds the vesicles to the organic frac-

tion of the soil thereby reducing leaching or runoff.

Another object of the present invention is to provide an encapsulator having better adhesion to plant cuticular waxes thereby preventing removal caused by rain or irrigation.

Still another object of the present invention is to reduce acute residue levels expected in plant tissue.

SUMMARY OF THE INVENTION

The invention is directed to preselecting a particular active agent such as a pesticide and an organic solvent for carrying the active agent. The same organic solvent must be effective to solubilize a plant lecithin to a lecithin-saturated level.

According to the invention, a plant lecithin is mixed with an organic solvent that is selected because it also dissolves the preselected active agent. The lecithin is present in a certain proportion so as to provide a solution with varied levels of lecithin saturation. In other words, an effective amount of lecithin is mixed in the organic solvent to form a saturated solution of lecithin. And it is well known that different organic solvents necessarily dissolve differing amounts of lecithin because of their varied levels of lecithin saturation from one organic solvent to the other.

The lecithin-saturation level will be dependent upon the amount of active agent (AA) desired in the final solution. That is, the organic solvent selected for dissolving the lecithin at its saturation level will also be effective to dissolve and otherwise carry the particular amount of AA desired in the final solution to be mixed with water to produce the vesicle formation.

Depending on the formulation of organic solvent and lecithin, the lecithin/organic solvent mixture is then

allowed to settle. That is, when the formulation produces a mixture having a saturated solution of lecithin and undissolved portions of the lecithin, the undissolved portions are allowed to settle to the bottom of the mixture leaving the solution at its lecithin-saturation level of the organic solvent.

After settling, the top layer of lecithin-saturated solution of the lecithin/organic solvent mixture is separated and saved, while the bottom layer is discarded. The lecithin-saturated solution is called the "stock solution" for the purpose of describing this invention. An AA is then added to the stock solution and the resulting concentrate is added to water for the agricultural application.

The resulting concentrate is an intermediate active agent solution and includes the lecithin-saturated solution plus the amount of AA required to be used in the prior art as a pesticide in the subsequent agricultural application. The invention is limited to dissolving a plant lecithin to be used in combination with the pesticide solubilized in the organic solvent thereby producing the desired initial predetermined lecithin-saturated stock solution.

The invention is directed to a method for preparing an intermediate active agent solution containing a preselected active agent and being effective to produce liposomal microencapsulation of the active agent by mixing the intermediate active agent solution with water.

An organic solvent solution is selected that is capable of carrying the preselected active agent in the solvent solution in an amount sufficient for agricultural applications.

The organic solvent solution is also capable of dissolving a preselected plant lecithin in an amount sufficient to produce in the solution an amount of phosphotidylcholine effective to encapsulate the pesticide when the intermediate

pesticide solution is mixed with water.

The preselected plant lecithin is mixed with the organic solvent solution to produce an initial predetermined lecithin stock solution containing the desired amounts of phosphotidylcholine in the organic solvent solution.

The predetermined lecithin stock solution produced in said process mixing step is isolated and then at the desired time, mixed with an amount of the preselected active agent to form the intermediate active agent solution having an active agent content sufficient for agricultural uses. The intermediate active agent solution is mixed with water forming an agricultural liquid formulation having the active agent encapsulated in a liposomal composition.

A particular feature of the invention is directed to the use of a pesticide mixed with a solution having a w/v or v/v ratio of lecithin to organic solvent in the solvent solution of 1:1 or 1:2. The method is limited to the use of a plant lecithin. In a specific embodiment, the organic solvent solution includes n-methyl pyrolidone and the plant lecithin has a phosphotidylcholine content in the range of from 5% to 50% of the lecithin.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The present application discloses a method of delivering an active agent onto plants, animals, structure surfaces, soils, and the like. The active agent is microencapsulated liposomally, thereby providing a delivery mechanism and a controlled release mechanism for the active agent.

In the present process, a plant lecithin is mixed with an organic solvent in a certain proportion so as to provide a solution at a desired lecithin-saturation level depending on the formulation. The amounts of a particular form of plant lecithin required to obtain a desired level of solubility in certain organic solvents are known to the skilled

artisan. The lecithin/organic solvent mixture is then allowed to settle. After settling, the top layer is separated and saved, while the bottom layer is discarded. An AA is then added to this stock solution and the resulting concentrate is added to water for the particular agricultural application. The concentrate must be added to water for vesicle formation.

Although, in the present invention, 100% denatured anhydrous ethanol may be used, it is only one of many solvents that can be used depending on the particular application. N-methyl pyrolidone (NMP) is used as a solvent in some applications. NMP is neither highly flammable nor carcinogenic and should pose little or no problem with the Environmental Protection Agency.

Other solvents that may be used are acetone, 100% denatured anhydrous ethanol, methylene chloride, 95% ethanol, 95% denatured ethanol, dimethylformamide, and gamma butyrolactone, to name a few. In practice, the solvents used with the active agents are generally known in the agricultural industry. Each producer or deliverer of the active agents generally knows which solvents can be used with its active agent.

Although any active agent can be used, the preferred embodiment uses Alachlor, Alphamethrin, Atrazine, Carbaryl, Chlorothalonil, Cymiazole, Cupric hydroxide, Cypermethrin, EPTC, Fluometuron, Lambda Cyhalothrin, Permethrin, Piperonyl Butoxide, Streptomycin, or Trifluralin. This list is not intended to be comprehensive but merely illustrative.

The skilled artisan in the agricultural industry knows the effective amounts of a particular pesticide to be used to accomplish its purposes in terms of pounds of pesticide per acre (lb/A) for the particular crop. The particular organic solvent for a desired pesticide is also known. Table I shows the known effective agricultural use rates and

solvents for known pesticides. In other words, the known agricultural use rate necessarily defines an amount of active agent sufficient for agricultural applications.

TABLE I

COMMON NAME	AGRICULTURAL USE RATE (lb/A)	SOLVENT
Herbicides:		
Alachlor	1.5 - 8	Soluble in ether, acetone, benzene, chloroform, ethanol, ethyl acetate, slightly soluble in heptane.
Atrazine	2 - 4	Dimethyl sulfoxide (18.3%), chloroform (5.2%), ethyl acetate (2.8%), methanol (1.8%), diethylether (1.2%), n-pentane (.035%), water (.0033%).
EPTC	2 - 6	Miscible in acetone, ethyl alcohol, kerosene, methyl isobutyl ketone, and xylene. Only .037% in water.
Fluometuron	.8 - 4	Soluble in dimethylformamide, acetone, ethanol, and isopropanol. Only .009% in water.
Trifluralin	.5 - 1	>50% soluble w/v in acetone, acetonitrile, chloroform, dimethylformamide, dioxane, hexane, methyl ethyl ketone, and xylene. 44% soluble w/v in methyl cellosolve, and .00003% in water. Formulated with xylene, ethyl benzene, and naphthalene.
Insecticides:		
Carbaryl	.5 - 2	Soluble in most polar organic solvents such as acetone. Only .004% in water.

Cypermethrin	.025 - .1	Soluble in methanol, acetone, xylene, and methylene dichloride. Insoluble in water.
Lambda cyhalothrin	.025 - .04	Soluble in most organic solvents. Low solubility in water. Formulated with xylene based petroleum solvent.
Malathion	.9 - 2.5	Soluble in most organic solvents. Only .0145% soluble in water. Formulated with xylene.
Permethrin	.05 - .4	Very soluble in most organic solvents except ethylene glycol. <.0001% soluble in water.
Piperonyl butoxide	.1 - .8	Soluble in most common organic solvents and petroleum distillates. Very slightly soluble in water.
Fungicides:		
Chlorothalonil	.56 - 4.13	Slightly soluble in organic solvents and insoluble in water.
Miscellaneous:		
Dyes & stains		Available in water soluble and solvent soluble forms.

Cymiazole and Benzocaine are used as active agents topically applied to animals, and Streptomycin is a bactericide used on fruit trees. In such agricultural applications, these pesticides are used in amounts measured in parts per million (ppm).

Cymiazole is an experimental insecticide, which is not registered in the United States. It is soluble to obtain an application amount of about 300 ppm of the solvent. It is 80% soluble in dichloromethane, 75% in methanol, 70% in toluene, 35% in octanol, 35% in NMP, 30% in isopropanol, 5%

hexane, and .005% in water. It is formulated in an aromatic solvent.

Streptomycin is used in an amount of about 100 ppm of the liquid for its agricultural use and is water soluble. Streptomycin is not very soluble in organic solvents.

Benzocaine is used for various agricultural uses and in a particular application on animals, in combination with Cymiazole. Generally, Benzocaine is used in an amount of about 1,000 ppm of the solution being used and is very soluble in ethanol and ether. It is insoluble in water.

N-methyl pyrolidone is an excellent general solvent. It dissolves most of the granulated plant lecithin constituents into it in the production of a NMP lecithin-containing stock solution. However, ethanol dissolves predominately the phosphotidylcholine (PC) in the production of an ethanol lecithin-containing stock solution. The other n-methyl pyrolidone soluble constituents in the n-methyl pyrolidone stock solution may prevent n-methyl pyrolidone from solubilizing PC to its capacity from the plant lecithin being used.

To overcome this problem, ethanol is first used to form a lecithin solution wherein the PC is extracted (solubilized) from the granulated or liquid lecithin into the lecithin solution. Then the ethanol is removed and the PC redissolved in the n-methyl pyrolidone. This helps to raise the PC content in the NMP stock. The extraction method does not add much to the total cost of the production process, but has extremely high PC levels. The extraction process could be done on any of the lecithins (high or low grade), depending on the desired result. Less expensive (14% PC) lecithins may be used as opposed to the more expensive (50% PC) granular lecithin depending on the desired result.

In mixing the lecithin and the solvent together, the preferred embodiment calls for a w/v ratio of lecithin to

solvent of 1:1 or 1:2, which is contrasted with the 1:3 as used in the prior art. This allows for the loading of high concentrations of AA into the stock, since it contains such high levels of PC.

The present process uses only one solvent in the formulation as a carrier for the lecithin and the AA. However, a "double solvent system" can be employed when certain active agents may not be soluble in a particular solvent that dissolves a considerable amount of lecithin. In this case, the AA may be dissolved in another solvent system, that may dissolve less lecithin but have a high capacity for the AA. The second solvent system may be used to dissolve the AA, while the addition to the first solvent system increases the amount of lecithin material into the solution, thereby ensuring the encapsulation of the AA. A double solvent system uses two, and the progression continues.

For each solvent system, the solvents are mixed with the lecithin before anything else. In a double solvent system, one will take two single solvent systems and mix them together. For example, for a double solvent system using solvents A and B, single solvent system A is produced when ethanol and a lecithin are mixed, and single solvent system B is produced when n-methyl pyrolidone is mixed with a lecithin. The AA would be added to the solvent system that had the higher solubility level for that particular AA.

The amounts of each single solvent system mixed together to form a double, triple or multiple solvent system, would vary depending upon the AA and what the particular solvent systems were.

More than one AA in a formulation can necessitate a solution requiring a triple solvent system. The addition of bulking agents such as methyl cellulose or Carbopol and other stabilizing agents required in some formulations could require a triple solvent system.

A large number of agricultural compounds degrade in the presence of ultraviolet light. The microencapsulation protects any AA enclosed within from the environment and ultraviolet light. Carotenoids are natural pigments that act as ultraviolet filters and are present in the lecithin material purchased from the manufacturer. High carotenoid concentrations are generally present in lecithin due to the lecithin extraction and refinement process.

The carotenoid concentration in the stock material may be regulated either by selective extraction or by total extraction from the lecithin material and then metering it back into the stock material. Regulating the carotenoid concentration would also regulate the rate of ultraviolet decomposition of the AA. Therefore, the length of time the AA will remain in the field can be controlled by the amount of the carotenoids in the lecithin.

Placement of a large amount of carotenoid in the system can protect the AA in the field for an extended period of time. Controlling the rate of release and breakdown of the encapsulated AA in this manner will also reduce the possible acute toxic effects when applied to crops or animals.

Depending on the AA used and its desired concentration in the final formulation, the PC content in the stock material may be selected for the AA by simple extraction techniques from less expensive lecithin materials with lower PC concentrations. The desired concentration of the AA in the final formulation is determined in accordance with the particular agricultural use rate established for the active agent such as the pesticides in Table I.

In accordance with the invention, first the organic solvent is selected to carry the necessary amounts of active agent to produce the required agricultural use rate in the final formulation of the encapsulated active agent. At the same time, the organic solvent selected for the active agent

must dissolve an effective amount of plant lecithin in solution to provide an intermediate active agent solution having a sufficient PC content for producing the vesicle formation of the liposomal encapsulation when the intermediate active agent solution is mixed with water.

Unlike the prior art, the most expensive lecithin is not required. Lecithin comes in different grades, depending upon the percentages of the components with phosphotidylcholine as the most important component. The higher the concentration of PC in the lecithin, the better the lecithin and the more expensive the process. Depending upon the active agent, the present method uses between five (5) to fifty (50) percent and, more particularly, fourteen (14) to fifty (50) percent PC lecithin.

The present invention can use a "batch" process to produce its stock solution. The production of the stock solutions under the present process may be automated to eliminate the slowness of the batch process. In doing so, the present invention utilizes augers and centrifuges to achieve the desired automation.

A system of a specific embodiment utilizes an auger to mix the lecithin and the solvent. As the mixing takes place the materials are moved down the auger leaving room for the addition of new unmixed materials on an automatically metered basis. As the materials become thoroughly mixed they are emptied into centrifuges for separation.

The centrifuges operate on a rotational basis. The rate of rotational speed of the centrifuges coincides with the flow rate of the auger and is designed to produce little or no backup in the system. The materials separated in the centrifuges are pumped into separate tanks for storage and disposal. Other processes such as those used in the alcohol distillery industry could also be utilized.

Due to the low flash point of ethanol and its extreme flammability all drive mechanisms for the system must be air-driven. The use of this technique in other industrial applications using ethanol has proven to be the most economic approach as opposed to providing a totally explosion proof electrical system. All compressors and other equipment for the pneumatic system would be located in other rooms or buildings away from the production area. The compressed air would be piped to each location.

Although any active agent can be used, in the preferred embodiment, the following active agents are used: Alachlor, Alphamethrin, Atrazine, Benzocaine, Carbaryl, Chlorothalonil, Cymiazole, cupric hydroxide, Cypermethrin, dyes, EPTC, Fluometuron, Lambda Cyhalothrin, Malathion, Permethrin, piperonyl butoxide, stains, Streptomycin, and Trifluralin.

Although any organic solvent can be used, in the preferred embodiment, the following organic solvents are used: acetone, 100% denatured ethanol, 95% denatured anhydrous ethanol, dimethylformamide, gamma butyrolactone, methylene chloride, and N-methyl pyrolidone.

In the preferred embodiment, the lecithin is selected from a group consisting of soybean oil, cottonseed oil, canola oil, wheat oil, kelp, peanuts, and sunflower seeds. The lecithin form is selected from a group consisting of liquid, granular, powder, gel, and paste. The lecithin has a PC content ranging from, but not limited to, 5% to 50% in the preferred embodiment. The PC content may be achieved by direct extraction, commercial preparation or custom blending.

Although any carotenoid content may be used, the preferred embodiment uses a carotenoid content from between substantially 0 to 1 percent to avoid active agent buildup. The carotenoid content may be achieved by direct extraction, commercial preparation or custom blending.

Commercially available plant lecithin is composed of the phospholipids phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidic acid (PA), carotenoids, and, depending on the grade, varying levels of oils, triglycerides, fibrous materials and, in some cases, additives and surfactants. Commercial lecithin is available in dry granular, liquid, gel, paste, and powder forms.

A solvent is selected for a particular stock material from available solvents that will dissolve the active agent and a compatible grade of lecithin and also be soluble in water. Different forms of plant lecithin (i.e. dry granular, liquid, gel, paste, and powder) have different levels of solubility in certain solvents. The form of lecithin chosen is thus dependent upon the solvent required to dissolve the active agent. For instance, if an active agent is soluble in ethanol then a lecithin form with low oil content such as a dry granular or gel would be selected because oily liquid lecithin forms are not very soluble in ethanol.

Flocculation problems sometimes arise when mixing with water due to an incompatibility of the solvent-active agent-lecithin form mixture. To overcome this problem, solvents and lecithin forms must be substituted with others to find the best combination for optimum suspensions in water. Also, when there is an active agent that is only soluble in hydrophobic nonpolar solvents, a co-solvent that is miscible in water and the nonpolar solvent must be introduced into the solvent system in order to mix with water.

Since lecithin is made up of many components, dissolving lecithin regardless of its form in a solvent will dissolve some of the components other than PC as well.

Specific examples of the invention are as follows:

Example I

An industry available liquid lecithin was mixed with n-

methyl pyrolidone (NMP) in a 1:2 v/v ratio. This mixture was stirred until it became visibly homogenized. It was then poured into a separatory funnel and allowed to settle. After sufficient time for settling took place (24-48 hr.), the bottom sludge fraction was drawn off and discarded while the top portion was decanted and saved as usable stock material.

This stock material is the primary carrier system for Chlorothalonil, the active agent to be added. The following formulation of one gallon provides an optimum suspension with minimal rapid settling when ultimately added to water to effect encapsulation.

Chlorothalonil technical (98% purity)	0.90 lbs
Anti-microbial agent	0.29 ozs (0.24 fl.oz.)
Anti-foaming agent	0.14 ozs (0.14 fl.oz.)
Thickening agent	1.43 ozs
Stock material	7.90 lbs (0.83 gal.)

Materials mixed with the concentrate add to stability and efficacy of the entire system. The anti-microbial agent retards bacterial decomposition of Chlorothalonil when applied to the field. The anti-foaming agent is added because the stock material has a tendency to foam when agitated which would hamper the concentration's ability to be accurately measured out for field application. Due to Chlorothalonil's insolubility, it settles out in the concentrate rapidly which necessitates the need for a thickening or suspending agent. The thickening agent is to be dispensed accurately.

A rate of 1.5 lb Chlorothalonil is required to spray an acre of tomatoes to control various fungal diseases. To

accomplish this using the Chlorothalonil concentrate of the invention, 1.7 gal of the concentrate was added to a water in a spray tank previously calibrated, according to standard practice, to deliver 20-40 gallons of finished aqueous spray solution to the acre.

Liposome formation is caused when the concentrate comes into contact with the water in spray tank. With the Chlorothalonil microencapsulated according to the invention, the longevity of the pesticide is extended so that it does not have to be applied as often as other Chlorothalonil products. Other new and unexpected results such as a significant increase in crop harvest have been obtained using the procedure of this invention in the field.

Example II

An agricultural industry available dry granular or powder lecithin was mixed with absolute ethanol in a 1:2 w/v ratio and agitated until all the lecithin was dissolved in the solvent. (For safety concerns, it is not recommended to heat mixtures for enhancing the dissolving of the solid material in volatile organic solvents such as ethanol.)

After all the lecithin dissolved, the mixture was transferred to a separatory funnel and allowed to settle. After sufficient settling time, the bottom sludge layer was drawn off and discarded and the top fraction saved as usable stock material.

Permethrin technical (92% purity) 1.02 lb

Stock material 6.48 lb (0.9 gal)

An average rate of 0.2 lb Permethrin per acre is usually required to control most insect problems in a variety of cropping situations. To accomplish this, 1.7 pints Permethrin concentrate of the invention was added to a previously calibrated spray tank, according to standard practice, to deliver 20-40 gal of finished aqueous spray solution to the acre.

Here again, liposome formation is caused when the concentrate comes into contact with the water in the spray tank. By microencapsulating, the effective longevity of Permethrin with its other unique features produce new and unexpected results in the field.

Example III

The stock material for Streptomycin was made in the same manner as for Permethrin using a 1:2 w/v ratio of lecithin to solvent. A 25% w/v Streptomycin concentrate was formulated to control fireblight in apples. A gallon of Streptomycin concentrate was made by bringing 2.09 lb of Streptomycin sulfate up to a gallon with stock material. The recommended rate to control fireblight in fruit trees is to prepare a 100 parts per million Streptomycin spray solution. Thus, 5.1 fl.oz. of the Streptomycin concentrate of the invention was added to 100 gallons of water in a calibrated air-blast orchard sprayer. On the average, this volume of spray solution will cover approximately an acre of

fruit trees.

While the METHOD OF PREPARING A PREDETERMINED ACTIVE AGENT STOCK SOLUTION FOR LIPOSOMAL MICROENCAPSULATION OF ACTIVE AGENTS FOR AGRICULTURAL USES has been shown and described in detail, it is obvious that this invention is not to be considered as limited to the exact form disclosed, and that changes in detail and construction may be made therein within the scope of the invention without departing from the spirit thereof.

CLAIMS

Having thus set forth and disclosed the nature of this invention, what is claimed is:

1. A method for the production of a predetermined stock intermediate pesticide solution containing a preselected pesticide and being effective to produce liposomal microencapsulation of the pesticide for agricultural applications by mixing the intermediate pesticide solution with water, said method comprising the steps of:

a) mixing an organic solvent capable of carrying the preselected pesticide with a plant lecithin to form a mixture of said solvent and plant lecithin with the lecithin being at a lecithin saturation level in said organic solvent;

b) settling said mixture to create a lecithin-saturated solution portion and an undissolved portion;

c) separating the lecithin-saturated solution portion from the undissolved portion for subsequent use of the lecithin-saturated solution in a later pesticide mixing step; and

d) mixing an amount of the preselected pesticide with the lecithin-saturated solution sufficient to form the intermediate pesticide solution that is effective for agricultural uses and for subsequent mixing of the pesticide with water to form liposomal encapsulation before an agri-

cultural application.

2. The method as defined in Claim 1 wherein said organic solvent is selected from a group consisting of acetone, 100% denatured anhydrous ethanol, 95% denatured ethanol, dimethylformamide, gamma butyrolactone, methylene chloride, and N-methyl pyrrolidone.

3. The method as defined in Claim 1 wherein said plant lecithin is obtained from a lecithin source selected from a group consisting of soybean oil, cottonseed oil, canola oil, wheat oil, kelp, peanuts, and sunflower seeds.

4. The method as defined in Claim 1 wherein said plant lecithin has a phosphotidylcholine content in the range of from about 5% to about 50%.

5. The method as defined in Claim 1 wherein said organic solvent is capable of carrying a pesticide selected from the group consisting of Alachlor, Alphamethrin, Atrazine, Carbaryl, Chlorothalonil, Cymiazole, cupric hydroxide, Cypermethrin, S-ethyl dipropylthiocarbamate, Fluometuron, Lambda Cyhalothrin, Permethrin, piperonyl butoxide, Streptomycin, and Trifluralin.

6. The method as defined in Claim 1 wherein

said organic solvent is capable of carrying a pesticide which first has been dissolved in a second organic solvent, and

said second organic solvent is capable of dissolving the plant lecithin therein.

7. A method for preparing a predetermined intermediate pesticide solution containing a preselected pesticide and being effective to produce liposomal microencapsulation of the pesticide for agricultural applications by mixing the intermediate pesticide solution with water, said method comprising the steps of:

a) selecting an organic solvent solution capable of carrying the preselected pesticide in said solvent solution in an amount sufficient for agricultural applications,

b) said organic solvent solution further being capable of dissolving a preselected plant lecithin in an amount sufficient to produce in the solution an amount of phosphotidylcholine effective to encapsulate the pesticide when the intermediate pesticide solution is mixed with water for said agricultural applications,

c) mixing said preselected plant lecithin with said organic solvent solution to produce a predetermined lecithin stock solution containing said amounts of phosphotidylcholine in the organic solvent solution,

d) isolating the predetermined lecithin stock solution produced in said process mixing step, and

e) mixing an amount of the preselected pesticide into said predetermined lecithin stock solution to form said intermediate pesticide solution having a pesticide content sufficient for agricultural uses.

8. The method as defined in Claim 7 wherein said predetermined lecithin organic solvent solution includes a mixture of a first organic solvent in which plant lecithin has been dissolved and a second organic solvent which is mixed with the first organic solvent to enhance the amount of phosphotidylcholine in said lecithin organic solvent solution.

9. The method as defined in Claim 8 wherein said first organic solvent is ethanol and said second organic solvent is n-methyl pyrrolidone.

10. The method as defined in Claim 7 wherein the w/v or v/v ratio of lecithin to organic solvent in said solvent solution is 1:1 or 1:2.

11. The method as defined in Claim 7 wherein the organic solvent solution includes n-methyl pyrrolidone.

12. The method as defined in Claim 7 wherein the plant lecithin is in a form selected from the group consisting of liquid, granular, powder, gel, and paste.

13. The method as defined in Claim 7 wherein the plant lecithin has a phosphotidylcholine content in the range of from about 5% to about 50% of the lecithin.

14. The method as defined in Claim 7 wherein said plant lecithin includes carotenoid.

15. A method for preparing an intermediate active agent solution containing a preselected active agent and being effective to produce liposomal microencapsulation of the active agent for agricultural applications by mixing the intermediate active agent solution with water, said method comprising the steps of:

a) selecting an organic solvent solution capable of carrying the preselected active agent in said solvent solution in an amount sufficient for agricultural applications,

b) said organic solvent solution further being capable of dissolving a preselected plant lecithin in an amount sufficient to produce in the solution an amount of

phosphotidylcholine effective to encapsulate the active agent when the intermediate active agent solution is mixed with water,

c) mixing said preselected plant lecithin with said organic solvent solution to produce an undissolved portion and an initial predetermined lecithin stock solution containing said amounts of phosphotidylcholine in the organic solvent solution, and

d) isolating said predetermined lecithin stock solution produced from said undissolved portion, and

e) mixing an amount of the preselected active agent into said initial predetermined lecithin stock solution to form said intermediate active agent solution having an active agent content sufficient for agricultural uses.

16. The method as defined in Claim 15 wherein said predetermined lecithin organic solvent solution includes a mixture of a first organic solvent in which plant lecithin has been dissolved and a second organic solvent which is mixed with the first organic solvent to enhance the amount of phosphotidylcholine in said lecithin organic solvent solution.

17. The method as defined in Claim 15 wherein the w/v or v/v ratio of lecithin to organic solvent in said solvent solution is 1:1 or 1:2.

18. The method as defined in Claim 15 wherein the organic solvent solution includes n-methyl pyrrolidone.

19. The method as defined in Claim 15 wherein the plant lecithin is in a form selected from the group consisting of liquid, granular, powder, gel, and paste.

20. The method as defined in Claim 15 wherein the plant lecithin has a phosphotidylcholine content in the range of from 5% to 50% of the lecithin.

21. The method as defined in Claim 15 wherein said plant lecithin includes carotenoid.

22. The method as defined in Claim 15 wherein said organic solvent solution is capable of carrying an active agent selected from the group consisting of Alachlor, Alphamethrin, Atrazine, Carbaryl, Chlorothalonil, Cymiazole, cupric hydroxide, Cypermethrin, S-ethyl dipropylthiocarbamate, Fluometuron, Lambda Cyhalothrin, Permethrin, piperonyl butoxide, Streptomycin, Trifluralin, Aenzocaine, dyes, Malathion, and stains.

23. A stock composition for mixing with water to

produce liposomal encapsulation of an active agent useful for agricultural formulations, said composition comprising:

a) an organic solvent carrying a preselected active agent in an amount sufficient for producing agricultural formulations,

b) said organic solvent including an amount of preselected plant lecithin sufficient to produce in the solvent an amount of phosphotidylcholine effective to encapsulate the preselected active agent when the stock composition is mixed for water to form said agricultural formulations,

c). said stock active agent for forming an intermediate active agent solution being effective to produce liposomal microencapsulation of the preselected active agent by mixing the intermediate active agent solution with water.

24. A method for producing liposomal microencapsulation of an active agent for agricultural uses, said method comprising the steps of:

a) selecting an organic solvent solution capable of carrying a preselected active agent in said solvent solution in an amount sufficient for agricultural applications,

b) said organic solvent solution further being capable of dissolving a preselected plant lecithin in an amount sufficient to produce in the solution an amount of

phosphotidylcholine effective to encapsulate said sufficient amount of active agent when an intermediate active agent solution is mixed with water,

c) mixing said preselected plant lecithin with said organic solvent solution to produce an undissolved portion and an initial predetermined lecithin stock solution containing said effective amount of phosphotidylcholine in the organic solvent solution, and

d) isolating said predetermined lecithin stock solution produced from said undissolved portion,

e) mixing an amount of the preselected active agent into said initial predetermined lecithin stock solution to form said intermediate active agent solution having said preselected active agent in said amount sufficient for agricultural uses, and

f) mixing said intermediate active agent solution with water to produce liposomal encapsulation of said active agent in said amount sufficient for agricultural applications.

25. The method as defined in claim 24 wherein said predetermined lecithin stock solution includes a mixture of a first organic solvent in which plant lecithin has been dissolved and a second organic solvent which is mixed with the first organic solvent to enhance the amount of phosphotidylcholine in said lecithin stock solu-

tion.

26. The method as defined in claim 24 wherein the w/v or v/v ratio of lecithin to organic solvent in said solvent solution is 1:1 or 1:2.

27. The method as defined in claim 24 wherein the organic solvent solution includes n-methyl pyrrolidone.

28. The method as defined in claim 24 wherein the plant lecithin is in a form selected from the group consisting of liquid, granular, powder, gel, and paste.

29. The method as defined in claim 24 wherein the plant lecithin has a phosphotidylcholine content in the range of from 5% to 50% of the lecithin.

30. The method as defined in claim 24 wherein said plant lecithin includes carotenoid.

31. The method as defined in claim 24 wherein said organic solvent solution is capable of carrying an active agent selected from the group consisting of Alachlor, Alphamethrin, Atrazine, Carbaryl, Chlorothalonil,

Cymiazole, cupric hydroxide, Cypermethrin, S-ethyl dipropyl-thiocarbamate, Fluometuron, Lambda Cyhalothrin, Permethrin, piperonyl butoxide, Streptomycin, Trifluralin, Aenzocaine, dyes, Malathion, and stains.

32. The method as defined in claim 24 wherein said active agent is a pesticide.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/06572

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 9/127
US CL : 424/450; 264/4.1, 4.3
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/450; 264/4.1, 4.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NONE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,269,979 (FOUNTAIN) 14 DECEMBER 1993, abstract, column 1, line 30 through column 3, line 68, column 4, line 31 and examples 8 and 10.	1-32
Y	US, A, 5,277,914 (SZOKA) 11 JANUARY 1994, column 9, line 24 through column 10, line 9.	1-32
Y	D. Glick, ed., "METHODS OF BIOCHEMICAL ANALYSIS", Volume 33, published 1988, J. Wiley & Sons (New York, NY), pages 337-462, especially pages 350-352, 356-357, 359 and 362.	1-32

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 JULY 1995	13 OCT 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer G.S.KISHORE
Facsimile No. (703) 305-3230	Telephone No. (703) 308-2351

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